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Stabilization of beneficial traits in *Heterorhabditis bacteriophora* through creation of inbred lines

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Abstract

Serial culturing of organisms used for biological pest suppression often leads to detrimental genetic changes and loss of utility. We established that genetically homozygous inbred lines can deter beneficial trait decline in the entomopathogenic nematode, *Heterorhabditis bacteriophora*. Three inbred lines and the foundation population were serially cultured in the insect host, *Galleria mellonella*. Trait stability was evaluated by comparing serially cultured with non-cultured populations. Laboratory data indicated that serial culture of the foundation population (16 passages) resulted in more than a 30% loss in traits deemed beneficial for biological pest suppression i.e., virulence to an insect host (*Diaprepes abbreviatus*), reproductive capacity, heat tolerance (at 38 °C), and host-seeking ability. In contrast, the three inbred lines were impervious to decline in all beneficial traits. A greenhouse test targeting *D. abbreviatus* provided additional evidence that the biocontrol efficacy of the inbred lines remained stable during serial culture. Our results indicate that genetic factors played an important role in trait change, and creation of inbred lines may be a useful technique for maintaining beneficial traits.
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Keywords: Biological control; Entomopathogenic nematode; *Heterorhabditis*; Inbred line; Insect; Trait stability

1. Introduction

Serial culturing of organisms in a laboratory or industrial setting can lead to detrimental genetic changes and loss of utility (Hopper et al., 1993; Radwan et al., 2004). The phenomenon is widely documented in arthropod and microbial organisms used for biological pest suppression. When these organisms are isolated from nature and reared in the laboratory, or mass-produced for commercial purposes, traits that are beneficial to their pesticidal efficacy may be reduced or lost due to genetic processes such as drift, inbreeding, or inadvertent selection (Hopper et al., 1993; Hoy, 1985; Roush, 1990). Trait changes may also arise from non-genetic factors such as poor nutrition and disease (Hopper et al., 1993). Reductions in traits such as longevity, fecundity, and virulence have been reported in biological control agents during laboratory culture (Dulmage and Rhodes, 1971; Geden et al., 1992; Hopper et al., 1993; Poe and Ennis, 1970).

Methodology to stabilize or prevent trait changes in biological control agents and other organisms is desirable. Roush (1990) suggested that homozygous inbred lines would deter harmful trait changes during serial culture because the lines would be impervious to certain genetic processes such as selection. Our objective was to test this hypothesis using the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar as the subject organism.

Heterorhabditid nematodes are biological control agents capable of suppressing a variety of economically important insect pests (Klein, 1990; Shapiro-Ilan and Gaugler, 2002). The nematodes kill their invertebrate hosts with the aid of the mutualistic bacteria, *Photorhabdus* spp. (Boemare, 2002). Nematode infective juveniles, the only free-living stage, enter hosts and release their symbiotic bacteria, which reproduce, and cause host death through septicemia. The nematodes complete 1–3 generations within the host. After 7–10 days infective juveniles begin to emerge to search out new hosts (Adams and Nguyen, 2002). Heterorhabditids are exclusively hermaphrodites in the first generation and a mix of males, females, and hermaphrodites occur in subsequent generations (Koltai et al., 1995; Strauch et al., 1994). Entomopathogenic nematodes are cultured for experimental or commercial purposes using in vivo or in vitro methods (Friedman, 1990; Shapiro-Ilan and Gaugler, 2002).

Serial culture of entomopathogenic nematodes has resulted in alterations in beneficial traits. Shapiro et al. (1996) reported a reduction in heat tolerance of *H. bacteriophora* (IS5 strain) under laboratory rearing conditions. Wang and Grewal (2002) reported rapid deterioration in environmental tolerance (to heat, desiccation, and UV) and reproductive potential for *H. bacteriophora* (GPS11 strain) during laboratory maintenance. Similar trait changes have been observed in other entomopathogenic nematodes species (e.g., steinernematids) (Gaugler and Campbell, 1991; Stuart and Gaugler, 1996).

To test our hypothesis (that inbred lines would deter trait changes), we created multiple inbred lines from an *H. bacteriophora* foundation population, subjected three inbred lines and the foundation population to serial culture, and compared the populations under laboratory conditions for various traits that are important to pest control efficacy (virulence, reproductive capacity, heat tolerance, and host-seeking ability). Maintenance of stability in several biocontrol traits under laboratory conditions, however, may not necessarily translate into maintenance of pest control efficacy under less controlled conditions, where a multitude of traits are required for success. Therefore, we also compared pest control efficacy of the serially cultured inbred lines to the original foundation population under greenhouse conditions.

2. Materials and methods

2.1. Nematode strains and culture

Five *H. bacteriophora* isolates (Hb 2a-2, Hb 4a-1, Hb-VS, and Hb-V1 from Georgia, USA and Hb RU1 from New Jersey, USA) were collected for use in this study.

In the field, isolates were separated by a distance of at least 50 m. The isolates were collected from natural populations by baiting soil with the susceptible host, the greater wax moth, *Galleria mellonella* (L.) (Shapiro-Ilan et al., 2003). The nematode isolates were serially cultured for one or two passages through *G. mellonella* according to Kaya and Stock (1997). A genetically diverse foundation population was then created by combining 5000 infective juveniles of each isolate, and then infecting 10 *G. mellonella* with 500 infective juveniles; this was then repeated to generate sufficient material. Additional in vivo culturing for experimental purposes was accomplished in *G. mellonella* and nematodes were stored at 13 °C or in liquid nitrogen as necessary (Kaya and Stock, 1997). *G. mellonella* was chosen because it is the most common host of entomopathogenic nematodes used in mass in vivo production by industry and in laboratory studies (Shapiro-Ilan and Gaugler, 2002). All nematode culturing and experiments were conducted at 25 °C unless otherwise indicated.

2.2. Creation of inbred lines

An in vitro monoxenic culture of the foundation population was established on nematode growth media (NGM) (Sulston and Hodgkin, 1988) according to Lunau et al. (1993). Twenty-two inbred lines were created according to Glazer et al. (1991). Briefly, infective juveniles were surface sterilized and inoculated onto NGM that was pre-seeded with *Photorhabdus luminescens* (Thomas and Poinar). Developing nematode progeny were transferred individually to new media plates (one per plate), and next generation progeny were obtained from hermaphrodites. This process was repeated seven times resulting in inbred lines that were supposed to be 95% homozygous (Hartl and Clark, 1989; Sinnott and Dunn, 1939).

2.3. Evaluation of beneficial trait changes under laboratory conditions

The 22 inbred lines were passed through *G. mellonella* once and progeny (designated P[1]) were compared with the foundation population for virulence, reproductive capacity, and heat tolerance as described below. Subsequently, three of the inbred lines that did not differ from the foundation population in trait expression were randomly chosen for further study. The three inbred lines (lines 10, 12, and 21) were serially cultured along with the foundation population in *G. mellonella* for an additional 15 passages (= 16 total). For each passage and each line, 20 insects in individual dishes were each exposed to 1,000 infective juveniles.

Trait change in virulence, reproductive capacity, heat tolerance, and host-seeking among infective juveniles of the various populations was evaluated at three intervals,

i.e., after passage 6, 11, and 16 (P6, P11, and P16). To assess trait change in each evaluation trial, the serially cultured populations were compared with corresponding non-cultured populations (P3). The non-cultured populations were maintained (in liquid nitrogen) at two passages until just before the evaluation trials, at which time they were passed once (= P3) through *G. mellonella* in parallel with the serially cultured populations so that differences in nematode age would not be a factor. Thus, eight nematode populations were included in each of the three evaluation trials (serially cultured and non-cultured populations of the three inbred lines and the foundation population).

2.3.1. Virulence

Virulence of the infective juveniles against the diaprepes root weevil, *Diaprepes abbreviatus* (L.), was assessed according to Shapiro et al. (1999). This insect was chosen because it is one of the most important commercial targets for entomopathogenic nematode application (Shapiro-Ilan et al., 2002). *D. abbreviatus* larvae (ca. 8th instar) were supplied by Division of Plant Industry (Gainesville, FL, USA) and fed on an artificial diet until used. Plastic cups (30 ml) contained one larva and 27 g sand each. Approximately, 500 infective juveniles were added to each cup; controls received only water. Larval mortality was checked at 9 and 14 days after nematode application. There were five replicates of 10 cups (50 insects) for each treatment.

2.3.2. Reproductive capacity

To estimate reproductive capacity, eight nematode-killed insects obtained from virulence tests described above (at 9 days after nematode application) were weighed, and individually placed in White traps for collection of progeny (Kaya and Stock, 1997). All infective juveniles were collected until emergence ceased (ca. 30 days). The total number of infective juveniles produced was determined through dilution counts under a dissecting microscope (Kaya and Stock, 1997); there were eight replicates (individual cadavers) per treatment.

2.3.3. Heat tolerance

Differences in heat tolerance were evaluated by measuring nematode survival at 38 °C (Shapiro et al., 1996). Glass test tubes (9-ml capacity) containing 2 ml water were incubated for 1 h (shaking at approximately 80 rpm) in a 38 °C water bath. Approximately, 1200 infective juveniles in a 0.25 ml suspension were then added to each tube. After incubating for 3 h, one milliliter of suspension was placed into a 10-cm diameter plastic petri dish filled with 9 ml distilled water. After the dishes were incubated for an additional 24 h at 25 °C to permit recovery, survival was determined; there were four replicates (test tubes and petri dishes with nematode suspensions) per treatment.

2.3.4. Host-seeking ability

Host-seeking ability was evaluated based on procedures described by Grewal et al. (1994) using last instar *G. mellonella* as a host. Briefly, petri dishes (10 cm diameter) were filled about 2/3 in depth with 2% water agar. A pipette tip was inserted into a hole in the lid on opposite ends of the plate (approximately 4 mm from the plate edge), and a nematode inoculation port (1.5 cm diameter) was created in the center of the lid and sealed with duct tape. On the treatment side, one *G. mellonella* larva was placed in the pipette tip and the control side's pipette tip was left empty. The pipette tips were sealed with parafilm and a gradient of volatile host cues was formed over the next hour. Subsequently, approximately 1200 infective juveniles in 0.3 ml water were applied to an 8 mm filter paper disc (Whatman # 1) and inserted into the center of the agar dish through the 1.5-cm hole in the lid, which was then resealed with duct tape. After 1.5 h, host-seeking ability was calculated based on the percentage of infective juveniles found in a 2 cm circle under the treatment side pipette tip vs. the sum of those found in 2 cm circles under the treatment plus control tips. There were four replicates (petri dishes) per treatment.

2.4. Evaluation of inbred lines' biocontrol potential under greenhouse conditions

Pest control efficacy under greenhouse conditions (ca. 24–28 °C) was compared among the non-cultured foundation population and the three serially cultured inbred lines (i.e., lines 10, 12, and 21 after 16 in vivo passages). Nematodes were applied to plastic pots (15.2 cm depth, 16 cm diameter) at a rate of approximately 100 infective juveniles per cm². Each pot was filled with soil potting mix (Metro-Mix-360, Scotts Sierra Horticultural Products, Marysville, OH) and contained five *D. abbreviatus* larvae (ca. 8th–10th instar). Percentage *D. abbreviatus* survival was determined 14 days after treatment. There were five replicates of each of the four nematode treatments and an untreated control; the experiment was conducted twice (two trials).

2.5. Statistical analyses

Percentage data (mortality, survival, host-seeking, heat tolerance) were arcsine transformed (arcsine of square root), and reproduction counts were square root transformed, prior to analysis (Steel and Torrie, 1980). Initial evaluation of traits in the 22 inbred lines and the foundation population was accomplished through analysis of variance (ANOVA), and if F was significant ($P \leqslant 0.05$, SAS, 2001) treatment effects were elucidated through LSD. To evaluate trait change, treatment effects were analyzed through a two-way factorial analysis of variance (eight levels of population and three levels of

passage). When passage or the passage × population interaction was significant ANOVA was applied to each passage separately and treatment effects were further elucidated through LSD.

3. Results

3.1. Initial screening of inbred lines

Differences among the 22 inbred lines and the foundation population at P1 were detected (Fig. 1). Virulence to *D. abbreviatus* was higher in some inbred lines than others though none were significantly different from the foundation population ($F = 3.00$, $df = 23$, 72, $P = 0.0002$) (Fig. 1A). Certain inbred lines produced more infective juveniles in *D. abbreviatus* than other lines and the foundation population (e.g., the yield of some lines was approximately double the foundation population's yield), yet none of the lines produced less infective juve-

niles than the foundation population ($F = 2.43$, $df = 22$, 192, $P \leqslant 0.0001$) (Fig. 1B). Heat tolerance was higher in some inbred lines than in other lines and the foundation population (e.g., survival in line 16 was more than double the survival observed in the foundation population), and one inbred line (line #17) exhibited lower heat tolerance than the foundation population ($F = 14.14$, $df = 22$, 69, $P \leqslant 0.0001$) (Fig. 1C). The three inbred lines chosen for further study to assess susceptibility to trait changes (lines 10, 12, and 21) did not initially differ from the foundation population in any of the three traits tested (Fig. 1).

3.2. Evaluation of trait change

3.2.1. Effect of population, passage, and interaction

Evaluation of main effects in virulence, reproduction, heat tolerance, and host-seeking ability assays indicated a significant population and passage effect, but not a significant interaction except for heat tolerance (Table 1).
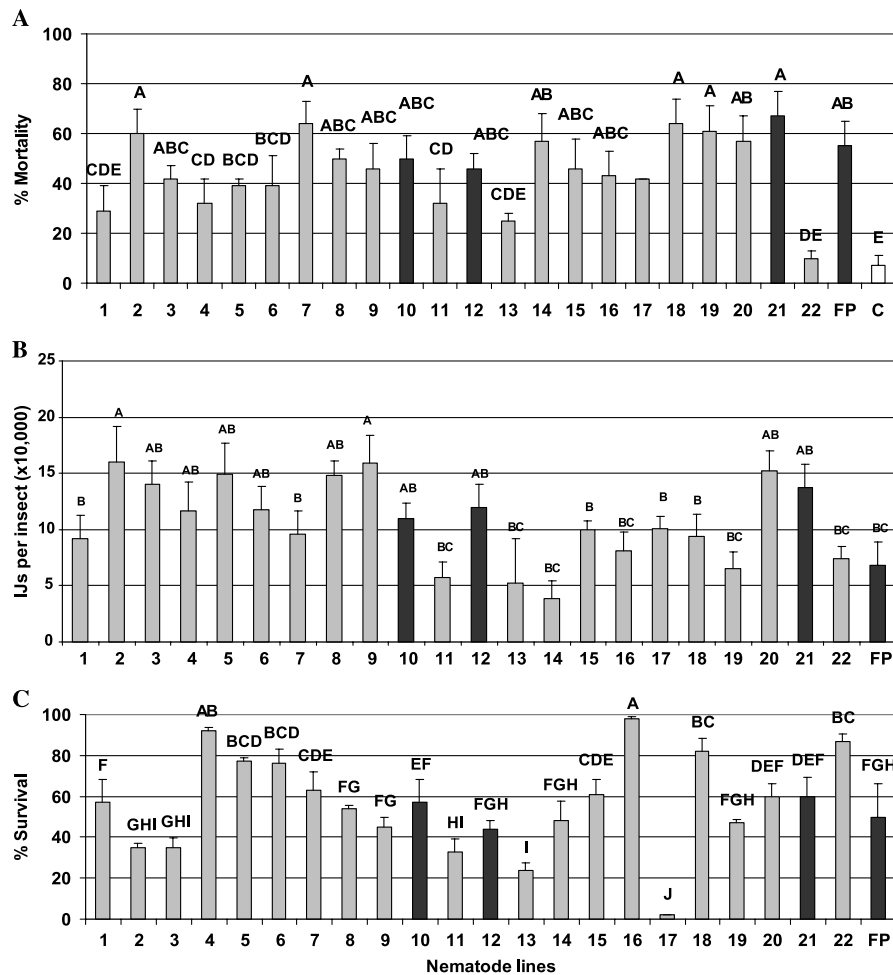

Fig. 1. Initial screening of *H. bacteriophora* inbred lines. Evaluation of 22 inbred lines and a foundation population for (A) virulence as indicated by mean percentage mortality (±SEM) of *D. abbreviatus* larvae, (B) reproductive capacity indicated by mean infective juveniles (IJs) (±SEM) produced per *D. abbreviatus* larva, and (C) heat tolerance measured by mean percentage nematode survival (±SEM) after exposure to 38 °C for 3 h. FP, foundation population; C, control (water only). Different letters above the bars indicate significant differences (based on LSD test, $P \leqslant 0.05$). Three inbred lines (# 10, 12, and 21) and the FP (bars filled in black) were chosen for further assay.

Table 1
Effect of population and passages on trait fitness among *Heterorhabditis bacteriophora* inbred lines and a foundation population (FP)[a]

Traits	Population			Passage			Interaction		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Virulence	9.89	7,96	$\leqslant 0.0001$	3.82	2,96	0.0253	1.31	14,96	0.2157
Reproduction	8.70	7,96	0.0001	4.01	2,96	0.0213	1.48	14,96	0.1349
Heat tolerance	7.37	7,72	$\leqslant 0.0001$	104.65	2,72	$\leqslant 0.0001$	3.62	14,72	0.0002
Host-seeking	3.42	7,72	0.0033	0.47	4,72	0.0496	0.99	14,72	0.4694

[a] Based on LSD test, $P \leqslant 005$.

Due to the significant passage effects in each trait, the treatment differences were analyzed separately at each passage (time period) for each trait (i.e., virulence, reproductive capacity, heat tolerance, and host-seeking ability).

3.2.2. Virulence

Virulence assays indicated trait changes in the serially cultured foundation population but not in the three inbred lines (Fig. 2). Within each inbred line or the foundation population, no differences in *D. abbreviatus* mortality were detected when P^3 was compared with the corresponding P^6 population; the only significant difference after six passages was that the foundation population caused less mortality than inbred line # 10 ($F = 3.76$, $df = 7$, 32, $P = 0.0044$). Similarly, no differences were observed between corresponding P^3 and P^{11} populations for each inbred line or the foundation population, yet all serially cultured (P^{11}) and non-cultured (P^3) inbred lines were more virulent than the foundation population at P^{11} ($F = 2.92$, $df = 7$, 32, $P = 0.0175$). After 16 passages the serially cultured foundation population had significantly lower virulence compared with the non-cultured foundation population and all of the inbred line populations ($F = 9.98$, $df = 7$, 32, $P \leqslant 0.0001$); no effects of serial culture were detected on virulence of the inbred lines after 16 passages.

3.2.3. Reproductive capacity

Serial culture resulted in reduced reproductive capacity in the foundation population, whereas the inbred lines remained stable (Fig. 3). No significant differences in reproductive capacity were detected when P^3 was compared with P^6 ($F = 0.68$, $df = 7$, 32, $P = 0.6910$). After 11 passages, significant differences were detected between corresponding serially cultured and non-cul-
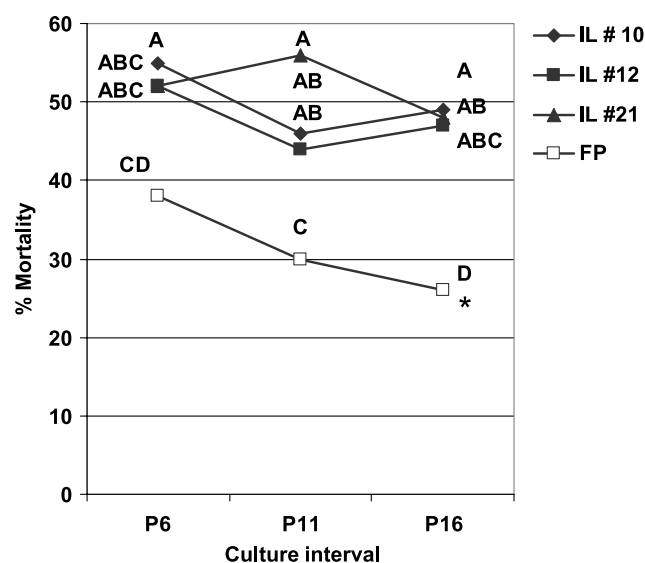
Fig. 2. Comparative virulence: mean percentage mortality of *D. abbreviatus* larvae exposed to three *H. bacteriophora* inbred lines (IL # 10, 12, and 21) and a foundation population (FP). Nematodes were serially cultured in *G. mellonella* larvae and compared with each other and with non-cultured populations at three intervals (i.e., after 6, 11, and 16 passages = P6, P11, and P16, respectively). Only means from serially cultured populations are displayed. Different letters above the lines indicate significant differences (LSD test, $P \leqslant 0.05$). *Indicates a significant difference between the serially cultured population and its corresponding non-cultured population.
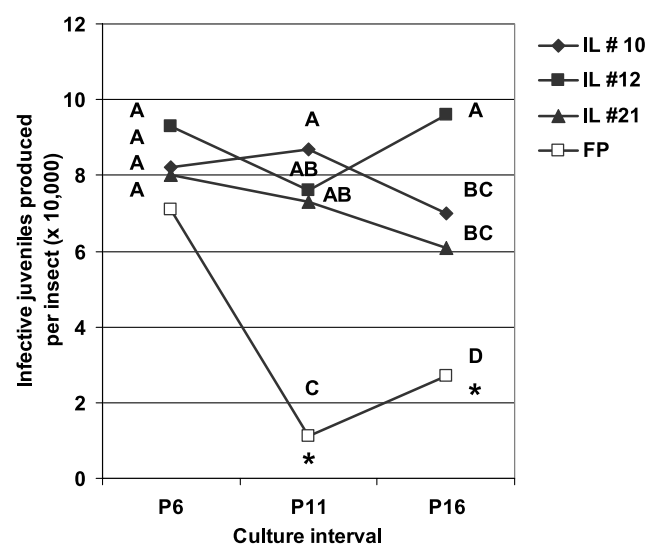
Fig. 3. Comparative reproductive capacity: mean number of infective juveniles produced per *D. abbreviatus* larva after infection with three *H. bacteriophora* inbred lines (IL # 10, 12, or 21) or a foundation population (FP). Nematodes were serially cultured in *G. mellonella* larvae and compared with each other and with non-cultured populations at three intervals (i.e., after 6, 11, and 16 passages = P6, P11, and P16, respectively). Only means from serially cultured populations are displayed. Different letters above the lines indicate significant differences (LSD test, $P \leqslant 0.05$). *Indicates a significant difference between the serially cultured population and its corresponding non-cultured population.

tured populations in the foundation population, but not in inbred lines; all inbred populations (P^3 and P^{11}) produced more nematode progeny than the serially cultured foundation population (P^{11}) ($F = 7.54$, $df = 7$, 40, $P \leqslant 0.0001$). At the 16th passage, no significant differences in progeny production were detected between serially cultured and non-cultured inbred lines; contrarily, reproductive capacity in the serially cultured foundation population was significantly reduced relative to the non-cultured foundation population and all of the inbred line populations ($F = 11.62$, $df = 7$, 32, $P \leqslant 0.0001$). Additionally, progeny production in line # 12 P^{16} was higher than in lines 10, 21, and the foundation population.

3.2.4. Heat tolerance

Serial culture of the foundation population resulted in a reduction of heat tolerance relative to the non-cultured foundation population and the inbred lines (which remained stable) (Fig. 4). No significant effects in heat tolerance were detected among serially cultured and non-cultured populations when comparing P^6 to P^3 ($F = 2.24$, $df = 7$, 24, $P = 0.0662$). After 11 and 16 passages, no significant effects in heat tolerance were detected between corresponding serially cultured and non-cultured inbred line populations; heat tolerance in the serially cultured foundation population, however, was significantly reduced relative to the non-cultured foundation population and all of the inbred line popula-

Fig. 5. Comparative host-seeking ability of three *H. bacteriophora* inbred lines (IL # 10, 12, and 21) and a foundation population (FP). The mean percentage of nematode infective juveniles that moved to an insect host (*G. mellonella*) was calculated. Nematodes were serially cultured in *G. mellonella* larvae and compared with each other and with non-cultured populations at three intervals (i.e., after 6, 11, and 16 passages = P6, P11, and P16, respectively). Only means from serially cultured populations are displayed. Different letters above the lines indicate significant differences (LSD test, $P \leqslant 0.05$). *Indicates a significant difference between the serially cultured population and its corresponding non-cultured population.

tions ($F = 7.18$, $df = 7$, 24, $P \leqslant 0.0001$ for P^{11}; $F = 8.17$, $df = 7$, 24, $P \leqslant 0.0001$ for P^{16}).

3.2.5. Host-seeking ability

Similar to the other traits tested, host-seeking ability declined in the foundation population during serial culture, whereas inbred lines remained stable (Fig. 5). No significant differences in percentage infective juveniles moving toward the host were detected between corresponding serially cultured and non-cultured populations after 6 ($F = 0.75$, $df = 7$, 24, $P = 0.6362$) or after eleven passages. After 11 passages the serially cultured foundation strain exhibited lower host-seeking ability than the inbred lines ($F = 2.52$, $df = 7$, 24, $P = 0.0430$). After 16 passages host-seeking ability in the serially cultured foundation population was significantly less than the non-cultured foundation population and all of the inbred lines (which did not differ from each other) ($F = 4.37$, $df = 7$, 24, $P = 0.0030$).

3.3. Evaluation of inbred lines' biocontrol potential under greenhouse conditions

No differences in biocontrol efficacy were detected between serially cultured inbred line populations and the non-cultured foundation population. Percentage survival of *D. abbreviatus* larvae was not different among pots
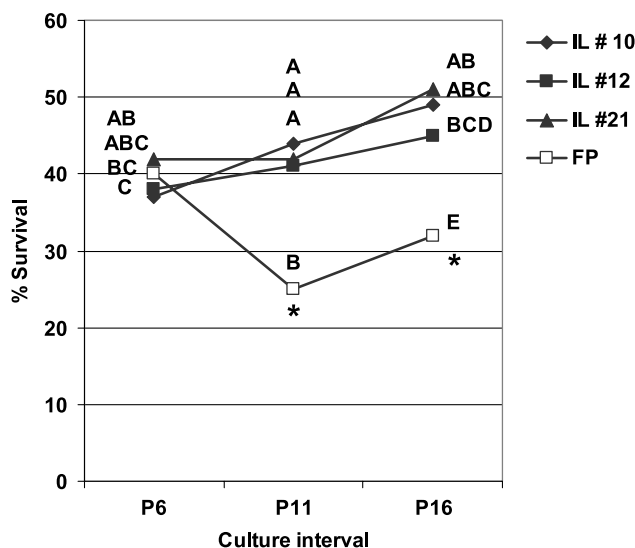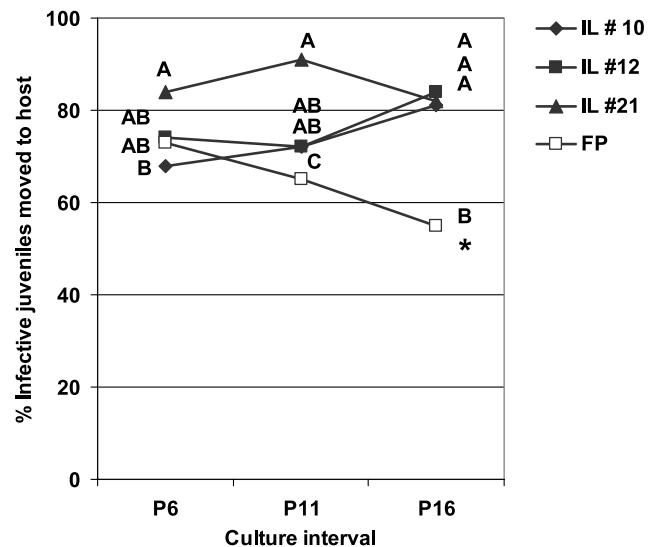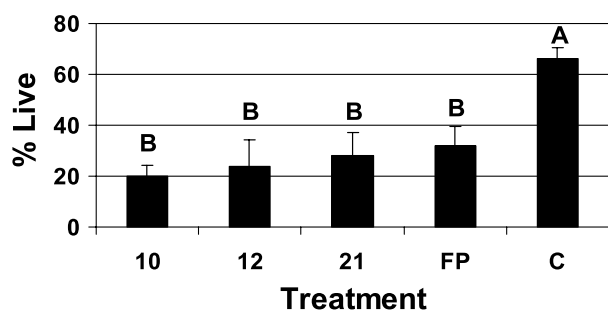

Fig. 4. Comparative heat tolerance: mean percentage survival of *H. bacteriophora* inbred lines (IL # 10, 12, and 21) and a foundation population (FP) after heat treatment for 3 h at 38 °C. Nematodes were serially cultured in *G. mellonella* larvae and compared with each other and with non-cultured populations at three intervals (i.e., after 6, 11, and 16 passages = P6, P11, and P16, respectively). Only means from serially cultured populations are displayed. Different letters above the lines indicate significant differences (LSD test, $P \leqslant 0.05$). *Indicates a significant difference between the serially cultured population and its corresponding non-cultured population.

Fig. 6. Mean percentage (±SEM) survival of *D. abbreviatus* larvae under greenhouse conditions after two weeks of exposure to soil treated with *H. bacteriophora* inbred lines (# 10, 12, or 21), a foundation population (FP), or a non-treated control (C). Inbred lines were serially cultured in *G. mellonella* larvae for 16 passages prior to the experiment. Different letters above the bars indicate significant differences (LSD test, $P \leqslant 0.05$).

treated with serially cultured inbred lines or the non-cultured foundation population, but all nematode treatments caused lower survival compared with the non-treated control ($F = 4.85$, $df = 4, 49$, $P = 0.0024$) (Fig. 6).

4. Discussion

We have established that inbred lines can prevent beneficial trait decline in the entomopathogenic nematode *H. bacteriophora*. For all traits tested, serial culture of the foundation population in vivo for 16 passages resulted in decline relative to the non-cultured (P^3) population and compared with the inbred lines. In contrast, all inbred lines remained stable.

For two traits, virulence and host-seeking, the serially cultured foundation population exhibited trait change relative to inbred lines at P^{11} before change was apparent in comparison with the non-cultured foundation population at P^{16}. We hypothesize that the foundation population changed slightly between P^1 and P^3, and therefore, to detect trait change a larger reduction in the serially cultured foundation population was required when making comparisons to the P^3 foundation population than when making comparisons to the inbred lines (we assume the inbred lines did not change between P^1 and P^3). Unfortunately, we could not test for trait changes between P^1 and P^3 in parallel, because P^1 was not maintained in the laboratory. Wang and Grewal (2002) reported trait deterioration in *H. bacteriophora* after only three passages, bolstering our hypothesis.

Our observation of trait change in the foundation population after 11–16 passages was anticipated. Previous observations of trait deterioration in *H. bacteriophora* occurred after three (Wang and Grewal, 2002) to eight (Shapiro et al., 1996) passages in *G. mellonella*. Trait changes in *Steinernema* spp. were reported after 12 and 20 passages (Gaugler and Campbell, 1991). For some other biological control agents, i.e., certain hyme-

nopteran parasitoids, trait deterioration was observed within four generations for some species, whereas others were reported only after 100 generations (Hopper et al., 1993). Factors that affect the rate of genetically based trait changes include the diversity and size of the reproducing population, the degree of selection pressure, and population or species differences (Bilgrami, Gaugler et al., unpublished data; Hopper et al., 1993; Stuart and Gaugler, 1996).

The deterioration observed in the foundation population (and withstood in the inbred lines) can be explained by genetic processes. Many studies reporting trait deterioration during laboratory culture of biological control agents attributed the alterations to genetic processes (e.g., drift, selection, inbreeding depression), yet relatively few have provided evidence (Hopper et al., 1993). Our study supports a genetically-based effect because if non-genetic processes (such as disease or poor nutrition) were the primary cause for the trait changes, then we would likely have observed change in the inbred lines as well as the foundation population. Studies with other biological control agents such as parasitic wasps have used inbreeding in an attempt to maintain beneficial traits (Koul and Dhaliwal, 2003; Sorati et al., 1996; Thomson et al., 2003), but ours is the first study as far as we are aware, to confirm the hypothesis and demonstrate stability in the inbred lines relative to mixed populations.

Maintenance of trait stability in inbred lines could be a valuable technique in laboratory culture and mass production of entomopathogenic nematodes. Not only are inbred lines more stable, but our results and those of others (Glazer et al., 1991) indicate that some inbred lines possess superior beneficial traits than their parent population. The stability of superior traits discovered in wild populations or produced through genetic improvement techniques (Gaugler, 1987) could be maintained through creation of inbred lines. These superior lines might be chosen by industry for development and commercialization. This approach could be powerful in particular for inundative applications where long-term persistence of the control agent is not expected or required, and reduced genetic variation and potential inability of inbred lines to adapt to the environment would not be a hindrance. It must be noted that, due to the multitude of traits required to succeed in pest suppression, some inbred lines that appear to be superior in several traits in the laboratory might be inferior under greenhouse or field conditions. Our greenhouse experiment, however, indicated that it is indeed feasible to develop inbred lines that maintain pest control abilities outside of the controlled conditions of the laboratory. Certainly, it is always recommended to verify laboratory results under greenhouse or field conditions. Yet even in cases where inbred lines are found to be inferior in field efficacy, two or more lines could be hybridized prior to

application to overcome weaknesses, as suggested by Roush (1990).

Acknowledgments

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